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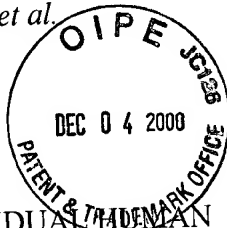
In re the Application of: D. Ward *et al.*

Serial No.: 09/335,956

Filed: June 18, 1999

For: DELINEATION OF INDIVIDUAL CHROMOSOMES IN METAPHASE AND INTERPHASE CELLS BY *IN SITU* SUPPRESSION HYBRIDIZATION

Attorney Docket No: IGI-001CN3



Group Art Unit: 1655

Examiner: Forman, B.

Assistant Commissioner for Patents  
Washington, D.C. 20231

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**AMENDMENT AND RESPONSE**

Dear Sir:

This is in response to the Office Action dated June 1, 2000 (Paper No. 8). A separate petition for the appropriate extension of time in which to respond is being filed concurrently herewith.

Please amend the application as follows:

In the Claims

Please cancel claims 8 and 11 without prejudice.

Please amend the remaining claims as follows:

1. (Amended) A method of labeling individual mammalian chromosomes [in mitotic cells or] in interphase cells by *in situ* hybridization, comprising:

providing [with] chromosome-specific labeled probes[,] and competitor DNA;

combining the labeled probes and competitor DNA with mammalian chromosomes in interphase cells under hybridization conditions wherein the labeled probes hybridize specifically to the mammalian chromosomes, thereby labeling the mammalian chromosomes in interphase cells [to produce a chromosome-specific signal].

2. (Amended) A method of labeling individual human chromosomes [in mitotic cells or] in interphase cells by *in situ* hybridization, comprising:

providing [with] chromosome-specific labeled probes[,] and competitor DNA;

combining the labeled probes and competitor DNA with human chromosomes in interphase cells under hybridization conditions wherein the labeled probes hybridize specifically to the human chromosomes thereby labeling the human chromosomes in interphase cells [to produce a chromosome-specific signal].

3. (Amended) A method of producing highly specific decoration of an individual human target chromosome in interphase cells, comprising:

providing [with] chromosome-specific labeled probes[,] and competitor DNA;

combining the labeled probes and competitor DNA with human chromosomes in interphase cells under hybridization conditions wherein the labeled probes hybridize specifically to the human chromosomes thereby producing highly specific decoration of individual human chromosomes [in situ suppression hybridization of labeled DNA probes, which are chromosome specific, to DNA in human mitotic cells or in human interphase cell].

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4. (Amended) The [A] method of [Claim] claim 3, wherein the [DNA] labeled probes are selected from the group consisting of probes comprising total recombinant library DNA, probes comprising DNA inserts purified from a chromosome-derived recombinant DNA library, and probes comprising specific DNA fragments derived from chromosomes.

5. (Amended) The [A] method of [Claim] claim 4, wherein the labeled [DNA] probes are selected from the group consisting of: [DNA] probes labeled with at least one fluorochrome[:]; [DNA] probes labeled with at least one member of a specific binding pair[:]; and [DNA] probes labeled with an enzyme.

6. (Amended) The [A] method of [Claim] claim 5, wherein the fluorochrome is selected from the group consisting of fluorescein, rhodamine, Texas red, Lucifer yellow, phycobiliproteins and cyanin dyes [and the member of a specific binding pair is biotin].

7. (Amended) A method of assessing [chromosome] chromosomal aberrations in human interphase cells by chromosomal *in situ* suppression hybridization, comprising:

providing labeled probes specific for human chromosomal aberrations and competitor DNA;

combining the labeled probes and competitor DNA with human chromosomes in interphase cells under hybridization conditions wherein the labeled probes hybridize specifically to the human chromosomes; and

detecting the labeled probes in order to assess chromosomal aberrations in human chromosomes in interphase cells.

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9. (Amended) A method of detecting chromosome aberrations in human aneuploid interphase cells, comprising:

treating [a] combining 1)) the human aneuploid interphase cells[, treated] so as to render nucleic acid sequences present in the human aneuploid interphase cells available for hybridization [with complementary nucleic acid sequences; and];

combining the treated human aneuploid interphase cells with [2)) a hybridization mixture comprising labeled human DNA derived from a specific chromosome[;] , competitor DNA[;] , and nonhuman genomic DNA, under conditions appropriate for hybridization of complementary nucleic acid sequences to occur; and [b)]

detecting labeled human DNA derived from the specific a chromosome [hybridized to nucleic acid sequences from the] in order to detect chromosome aberrations in aneuploid interphase cells.

10. (Amended) The [A] method of [Claim] claim 9, wherein the aneuploid interphase cells are human tumor cells.

12. (Amended) The [A] method of [Claim] claim 10, wherein the human tumor [cell is a] cells are human glioma [cell] cells.

13. A method of detecting [in a sample] numerical alterations in a human chromosome in interphase cells [present in the sample], comprising:

selecting a human chromosome;

treating interphase cells [a] combining 1) the sample, treated so as] to render nucleic acid sequences present in the cells [sample] available for hybridization [with complementary nucleic acid sequences; and 2)] ;

combining the interphase cells with a hybridization mixture comprising labeled human DNA derived from the selected chromosome; competitor DNA; and nonhuman genomic DNA, under conditions appropriate for hybridization of complementary nucleic acid sequences to occur; and [b)]

[detecting labeled DNA de-selected chromosome hybridization of complementary nucleic acid sequences to occur; and b)] detecting labeled DNA derived from the selected chromosome [hybridized to nucleic acid sequences present in the sample] in order to detect numerical alterations in a human chromosome in interphase cells.

14. (Amended) The [A] method of [Claim] claim 13, wherein the [selected] human chromosome is selected from the group consisting of [the following chromosomes:] chromosome 13, chromosome 18, chromosome 21, chromosome X and chromosome Y.

15. (Amended) The [A] method of [Claim] claim 13, wherein the [selected] human chromosome is chromosome [number] 21 and the labeled human DNA derived from the selected chromosome [is] comprises DNA inserts purified from a chromosome-derived recombinant DNA library.

16. (Amended) A method of determining over-representation or under-representation of a selected chromosome or a portion thereof in human tumor interphase cells comprising the steps of:

selecting a chromosome or portion thereof;

treating the [a] combining 1)] human tumor interphase cells[, treated so as] to render nucleic acid sequences present in the cells available for hybridization;

combining the human tumor interphase cells with [complementary nucleic acid sequences; and 2)] a hybridization mixture comprising labeled DNA fragments derived from [a] the selected chromosome[;] , competitor DNA[;] , and nonhuman genomic DNA, under conditions appropriate for hybridization of complementary nucleic acid sequences to occur; and

[b)] detecting labeled DNA fragments derived from the selected chromosome in order to determine the over-representation or under-representation of the selected chromosome or a portion thereof in human tumor interphase cells [human chromosome-specific DNA fragments hybridized to nucleic acid sequences from the tumor cells].

17. (Amended) A method of identifying chromosome-specific DNA present in a selected mammalian chromosome in interphase cells, comprising:

selecting a mammalian chromosome;

[a)] combining the [following substances: 1)] the selected mammalian chromosome[;] with [2)] labeled DNA fragments derived from the selected mammalian chromosome [bearing a detectable label; 3)], competitor DNA[;] and [4)] carrier DNA, under conditions appropriate for hybridization of complementary nucleic acid sequences to occur;

forming [to form] a complex of the labeled DNA fragments [bearing a detectable label] with the selected mammalian chromosome; and

[b)] detecting the complex of the labeled DNA fragments with the selected mammalian chromosome in order to identify chromosome-specific DNA present in a selected mammalian chromosome in interphase cells [complexes formed in step (a)].

18. (Amended) The [A] method of [Claim] claim 17, further comprising the step of separating the complex of the labeled DNA fragments with the selected mammalian chromosome [isolation of chromosome-specific DNA in a selected mammalian chromosome by separating the selected complexes formed] from the uncomplexed labeled DNA fragments, uncomplexed mammalian human chromosome, the competitor DNA and the carrier DNA [remaining substances combined in step (a)].

### REMARKS

Claims 1-18 were pending. Claims 8 and 11 have been canceled without prejudice. Accordingly, claims 1-7, 9, 10, and 12-18 are currently pending.

Claims 1-7, 9, 10, and 12-18 have been amended. In particular, claims 1-7, 9, 10, and 12-18 have been amended to recite positive active steps, as suggested by the Examiner. No new matter has been added.

The foregoing claim cancellations and amendments should in no way be construed as an acquiescence to any of the Examiner's rejections and was done solely to

expedite the prosecution of the application. Applicants reserve the right to pursue the claims as originally filed, or other claims of equivalent or broader scope, in another application(s).

***Rejection of Claims 1-18 Under 35 U.S.C. § 112, Second Paragraph***

Claims 1-18 are rejected under 35 U.S.C. § 112, second paragraph, "as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention."

Claims 8 and 11 have been canceled without prejudice. Claims 1-7, 9, 10, and 12-18 have been amended, as suggested by the Examiner, to recite positive active steps. Accordingly, the claims as amended are clear and indefinite. therefore, Applicants respectfully request the Examiner to withdraw the rejection of the claims under 35 U.S.C. § 112, second paragraph.

***Rejection of Claims 1-9, 13-15, and 17 Under 35 U.S.C. § 102(e)***

Claims 1-9, 13-15, and 17 are rejected under 35 U.S.C. § 102(e) "as being clearly anticipated by Gray et al. (U.S. Patent No. 5,447,841, filed 14 December 1990)."

Claim 8 has been canceled without prejudice. Therefore, the rejection of claim 8 is moot. With respect to claims 1-7, 9, 10, 13-15, and 17, Applicants respectfully traverse this rejection.

Claims 1-9, 13-15, and 17 are drawn to methods encompassing the analysis of chromosomes in *interphase cells* by means of chromosomal *in situ* suppression hybridization. Such methods include the use of chromosome-specific labeled probes, competitor DNA, and interphase cells comprising the chromosomes of interest. Gray *et al.* fail to teach or suggest such methods with sufficient description so as to enable one of ordinary skill in the art to practice the claimed methods without undue experimentation. Therefore, Gray *et al.* fail to anticipate the claimed methods.

While Applicants are aware that the Gray patent claims are broad, in assessing a patent's prior art effect, its disclosures are considered, not what may fall within its claims.

*In re Benno*, 768 F.2d 1340, 226 USPQ 683 (Fed. Cir. 1985) [emphasis added]. For reasons discussed in further detail below, the teachings of the Gray *et al.* patent do not anticipate the claimed invention.

Gray *et al.* discuss that the success of any particular *in situ* hybridization methods depends upon several critical factors. In particular, Gray *et al.* state (at col. 11, lines 16-23) that

[t]hree factors influence the staining sensitivity of a heterogeneous mixture of hybridization probes: (1) efficiency of hybridization (fraction of target DNA that can be hybridized by probe), (2) detection efficiency (*i.e.*, the amount of visible signal that can be obtained from a given amount of hybridization probe), and (3) level of noise produced by nonspecific binding of the probe or components of the detection system.

Gray *et al.* go on to discuss the numerous reagents and conditions that must be selected and optimized for any particular *in situ* hybridization technique (see col. 11, line 24 through col. 13, line 11). These include:

- type of fixative and fixation procedure;
- type of agent(s) for removing proteins and procedure for deproteinization;
- procedure for removal of residual RNA from fixed chromosomes;
- conditions for denaturation of chromosomal DNA so that probes can gain access to complementary single stranded regions;
- procedure for removal of denaturing agents;
- conditions for application of the heterogeneous probe mixture which allow the probes to anneal to complementary sites (*i.e.*, hybridization conditions);
- selection of the concentration of probes in the heterogeneous mixture;
- procedures for reducing non-specific binding of probe DNA;
- conditions for posthybridization washes to remove probe not bound to specific hybrids;
- conditions for detecting the hybridized probes.

While Gray *et al.* discuss these factors and provide some guidance as to reagents and conditions that may be used, Gray *et al.* admit numerous times throughout the specification that further experimentation may be required for particular applications. For example, Gray *et al.* state that

[t]he following comments are meant to serve as a guide for applying the general steps listed above. Some experimentation may be required to establish optimal staining conditions for particular applications [see col. 11, lines 36-39] . . .

Optimization of deproteinization requires a combination of protease concentration and digestion time that maximize hybridization, but does not cause unacceptable loss of morphological detail. Optimum conditions vary according to chromosome types and method of fixation. Thus, for particular applications, some experimentation may be required to optimize protease treatment [see col. 11, line 63 to col. 12, line 2]. . .

Determination of the optimal incubation time, concentration, and temperature within these ranges [for hybridization conditions] depends on several variables, including the method of fixation and the chromosome type [see col. 12, lines 27-30]. . .

Optimal hybridization conditions for particular applications depend on several factors, including salt concentration, incubation time of chromosomes in the heterogeneous mixture, and the concentrations, complexities and lengths of the probes making up the heterogeneous mixture. Roughly, the hybridization conditions must be sufficiently denaturing to minimize nonspecific binding and hybridizations with excessive numbers of base mismatches. On the other hand, the conditions cannot be so stringent as to reduce hybridizations below detectable levels or to require excessively long incubation times [see col. 12, lines 42-55].

For application of *in situ* suppression hybridization in interphase cells which uses competitor DNA, Gray *et al.* provide no specific guidance with regard to the myriad factors discussed above. Gray *et al.* disclose a single exemplification of chromosome-specific staining using competitor DNA containing repetitive sequences (see Paragraph VI at col. 15, line 58 through col. 16, line 57) and this exemplification uses *metaphase cells*. The specification provides no exemplification of interphase cells, no guidance as to whether the conditions used for metaphase cells are applicable to interphase cells, or whether certain conditions should be altered and, if so, how to alter them. At most, the Gray *et al.* patent provides merely a starting point for further experimentation on *in situ* suppression hybridization in interphase cells, using competitor DNA containing repetitive sequences.

In addition to the Gray *et al.* disclosure itself, there is additional evidence in the art that the ordinarily skilled artisan would not believe that Gray *et al.*'s exemplification

of *in situ* suppression hybridization in metaphase cells would enable the method in interphase cells, without further experimentation. In particular, Applicants draw the Examiner's attention to Landegent *et al.* (1987) *Human Genetics* 77:366-370 (of record as reference "CP"). Landegent *et al.* teach *in situ* hybridization in metaphase cells using as probes one or more cosmids of the human Tg gene. Landegent *et al.* (at page 367, column 2, second paragraph of the Results section) describe the probes used in their method as follows:

four different randomly chosen cosmids of the human Tg gene (of which the chromosomal localization is known, Baas *et al.* 1985; Landegent *et al.* 1985b) were used (Van Ommen *et al.* 1983; Baas *et al.* 1986) . . . These cosmids have not been characterized in the actual number and type of repeats present, but all gave a strong overall staining when hybridized to total human DNA under standard conditions.

Landegent *et al.* teach that the method described therein is not applicable to interphase cells. In particular, Landegent *et al.* (at page 369, column 2, last paragraph) state:

The method described here can extend the applicability of non-radioactive procedures since it elegantly bypasses the lower sensitivity problem, obviating subcloning of a minimal amount of unique parts required. When, in time, the sensitivity has reached a level allowing the detection of small (1-2 kb) single-copy sequences on metaphase chromosomes on a routine basis, the described procedure may still retain its attractiveness for several purposes. For example: . . . Detection of chromosomal aberrations in prenatal diagnosis, for example a trisomy, in interphase nuclei in microscopic slides (Cremer *et al.* 1986) or through hybridization in suspension and flow cytometry. In these cases it would be favorable to use a whole panel of chromosome-specific cosmids instead of a small unique probe or a cloned alphoid sequence to ensure strong hybridization signals with great specificity, for a more reliable diagnosis [emphasis added].

The above-quoted passage contains the only reference to interphase cells, or nuclei thereof, in Landegent *et al.* and it is entirely future-oriented ("when, in time..."). Thus, Landegent *et al.* clearly teach that the sensitivity of their method (*i.e.*, *in situ* suppression

hybridization in *metaphase cells*) is not sufficient for application to interphase cells. Accordingly, since Gray *et al.* essentially exemplify the same method as Landegent *et al.*, namely, *in situ* suppression hybridization in *metaphase cells*, and Landegent *et al.* teach that this method is not applicable to interphase cells, or nuclei thereof, without further experimentation, the ordinarily skilled artisan would not believe that Gray *et al.* had enabled this method for interphase cells.

In contrast to Gray *et al.*, Applicants have enabled a method of *in situ* suppression hybridization for detecting target chromosomal DNA *in situ* in *interphase cells* and exemplified this method in different cell types and using probes for detecting different target chromosomal DNAs, thereby demonstrating the general applicability of the claimed method. For example, in Example 1 (see, e.g., Figure 5), Applicants successfully detect chromosomes 1, 7 and 18 in normal lymphocyte interphase nuclei and in Example 2 (see, e.g., Figures 10B-10F), Applicants successfully detect chromosomes 1, 7 and 18 in interphase nuclei of tumor cells (glioma cells). Other successful demonstrations of the method in interphase cells, are found throughout the specific examples of the invention.

Since Gray *et al.* fail to enable the methods of 1-7, 9, 10, 13-15, and 17, these claims are not anticipated by Gray *et al.* Accordingly, Applicants respectfully request the Examiner to withdraw the rejection of claims 1-7, 9, 10, 13-15, and 17 under 35 U.S.C. §102(e).

***Rejection of Claims 10-12, 16, and 18 Under 35 U.S.C. § 103(a)***

Claims 10-12, 16, and 18 are rejected under 35 U.S.C. § 103(a) "as being unpatentable over Gray *et al.* (U.S. Patent No. 5,447,841, filed 14 December 1990)." Applicants respectfully traverse this rejection.

Claims 10-12, 16, and 18 are drawn to methods encompassing the analysis of chromosomes in *interphase cells* by means of chromosomal *in situ* suppression hybridization. Such methods include the use of chromosome-specific labeled probes, competitor DNA, and interphase cells comprising the chromosomes of interest. As discussed above, the substance of which is reiterated here, Gray *et al.* fail to teach or

suggest the pending claims. In particular, the teachings of Gray *et al.* do not enable *in situ* suppression hybridization in *interphase cells*.

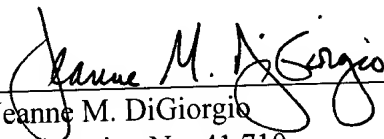
Furthermore, as discussed above, the teachings of Gray *et al.* fail to motivate or provide a reasonable expectation of success to one of ordinary skill in the art to make the claimed invention as evidenced by Landegent *et al.* Therefore, Applicants respectfully request the Examiner to withdraw the rejection of claims 10-12, 16, and 18 under 35 U.S.C. § 103(a).

### SUMMARY

In view of the foregoing, reconsideration of the rejections and allowance of the pending claims is requested. If a telephone conversation with Applicants' Attorney would expedite prosecution of the above-identified application, the examiner is urged to call the undersigned at (617) 227-7400.

Respectfully submitted,

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Dated: 1 December 00